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14. ABSTRACT

The purpose of this HBCU/MI Partnership Training Award was to train Meharry Medical College (MMC) faculty to conduct independent breast cancer research by collaborating with faculty from Vanderbilt University Medical Center (VUMC). Year 1 was a training year and during Years 2 through 4 a case-control study of obesity, insulin resistance and mammographic breast density was conducted. A no cost extension through 9 months of Year 5 was used to conduct final analyses, and disseminate results to researchers and participants. Specific aims included: 1) to assess mammographic breast density through digital mammograms; for a sample of women we will also assess mammographic breast density through film mammograms to determine the diagnostic accuracy of digital versus film mammogram, 2) to obtain information on breast cancer risk factors including health literacy, and to collect anthropometric measurements and fasting blood, 3) to assay blood for select hormones and growth factors, 4) to perform statistical analyses to determine the associations between obesity and insulin resistance and mammographic breast density, and 5) to evaluate patients' ability to understand their mammogram findings as they are explained by their medical provider. Subject recruitment; data collection through questionnaires, body measurements and digital mammograms; blood collection, processing and assaying for hormones, growth factors and adipokines; quality assurance, interim and final analyses of data; and dissemination of results as a published manuscript and as a newsletter was completed on 476 women. MMC investigators attended/presented at conferences and, in collaboration with VUMC investigators, published three manuscripts and produced a lay version of results for participants.

15. SUBJECT TERMS

Epidemiology/biostatistics, hormone metabolism

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Introduction

The purpose of this HBCU/MI Partnership Training Award was to train Meharry Medical College (MMC) faculty to conduct independent breast cancer research by collaborating with faculty from Vanderbilt University Medical Center (VUMC). Three MMC faculty underwent intensive training supervised by three VUMC faculty during year 1 with additional training taking place in subsequent years. To reinforce training, faculty from MMC and VUMC conducted a case-control study of mammographic breast density to investigate its' association with obesity and insulin resistance in years 2 through 5. Cases whose breasts were in the upper quartile of breast density and controls whose breast were in the lowest three quartiles of breast density, were recruited from the MMC Center for Women's Health Research which serves a medically underserved population. Specific aims were: 1) to assess mammographic breast density through digital mammograms; for a sample of women we also planned to assess mammographic breast density through film mammograms to determine the diagnostic accuracy of digital versus film mammogram, 2) to obtain information on breast cancer risk factors including health literacy, and to collect anthropometric measurements and fasting blood, 3) to assay blood for select hormones and growth factors, 4) to perform statistical analyses to determine the associations between obesity and insulin resistance and mammographic breast density, and 5) to evaluate patients' ability to understand their mammogram findings as they were explained by their medical provider.

Body

Dr. Maureen Sanderson replaced Dr. Alecia Fair as MMC Principal Investigator (PI) of the project effective July 11, 2011. As indicated in the Statement of Work (Appendix), this project occurred in two phases, the training phase (year 1) and the investigation phase (years 2 through 4). We completed all training tasks during the first year of the project; however, ongoing training tasks included the attendance and presentation of MMC investigators at workshops and conferences, the publication of a manuscript utilizing existing data, and Institutional Review Board (IRB) approval of the Mammographic Breast Density Project. We received a no cost extension on June 17, 2014 to extend the period of performance through June 30, 2015. We completed nearly all investigation tasks during years 2 through 5 of the project; however, we did not complete investigation task 6 by comparing analog and digital mammograms because it was beyond the scope of the study. During years 2 through 4 of the project the study team met on a monthly basis and the investigative team (Drs. Maureen Sanderson, Corey Jones/Heather O'Hara, and Waseem Khoder/Nia Foderingham from MMC and Drs. William Dupont, Xiao Ou Shu and Neeraja Peterson from VUMC) met on a quarterly basis. Currently, we are completing analyses and manuscripts on obesity, growth factors and adipokines, and health literacy as they relate to mammographic breast density.

During the first year of the project, we partially completed training task 1a by Dr. Sanderson attending the American Association for Cancer Research (AACR) Cancer Health Disparities Conference, and by presenting posters at the American Public Health Association Conference, the Society for Epidemiologic Research Conference, and the Department of Defense Era of Hope Conference; by Dr. Jones presenting a poster at the Clinical and Translational Science Award Community Engagement Conference; and by Dr. Khoder attending the AACR Advances in Breast Cancer Research Conference. We partially completed training task 1b by Dr. Jones taking Epidemiology, Fundamental Principles of Human Research, Biostatistics, Social and Behavioral Science for Public Health, Research Ethics, Molecular Medicine,

Communications/Grant Writing, and Clinical Trials in the Master's of Science in Clinical Investigation (MSCI) Program. We completed training task 1c by meeting with Drs. Richard-Davis, Disher, Al-Hendy and Mouton from the MMC Center for Women's Health Research to design the breast density study to include digital mammogram assessment, completion of a questionnaire, anthropometry and a blood draw. We completed training tasks 1d through 1l by developing a questionnaire appropriate for use with the local population; designing the protocols for subject recruitment, data collection, laboratory work, tracking system, data entry programs, and by writing the manual of operations. We obtained IRB approval initially from MMC on 9/7/2010, VUMC on 6/7/2011, and the Department of Defense (DOD) on 6/27/2011. Drs. Dupont, Shu and Peterson from VUMC provided input on the poster presented at the Era of Hope Conference and the questionnaire.

During the second year of the project we moved from the training phase into the investigation phase. Dr. Jones left MMC and was replaced by Dr. Heather O'Hara, a Preventive/Occupation Medicine physician with a Master's of Science in Public Health. Dr. Sanderson presented a poster at the Society for Epidemiologic Research conference and has submitted a manuscript from the poster for review, and Dr. Khoder attended the American Society for Clinical Oncology conference. We obtained continuing IRB approval for the project from MMC on 8/24/2012, VUMC on 5/1/2012, and DOD on 8/24/2012. Between January and September, 2012 we completed subject recruitment and data collection of 244 women. We partially completed investigation tasks 2 through 5 by quantitating mammographic breast density measurement; recruiting subjects and collecting data; assessing health literacy; and processing blood samples, taking body measurements and performing assays. We partially completed investigation tasks 7 and 8 by conducting ongoing quality assurance audits to ensure patient safety and integrity, and conducting interim analyses.

During the third year of the project we continued in the investigation phase. Dr. Sanderson attended the American Public Health Association conference, Dr. O'Hara attended the American College of Preventive Medicine conference, and Dr. Khoder attended the American College of Obstetrics and Gynecology conference. Using data from Dr. Sanderson's previous study (DAMD17-03-1-0274), Drs. Sanderson, O'Hara and Khoder presented a poster at the Research Centers in Minority Institutions International Symposium on Health Disparities and published a manuscript (Appendix). We obtained continuing IRB approval for the project from MMC on 8/19/2013, VUMC on 3/18/2013, and DOD on 9/9/2013. Between October 12, 2012 and October 11, 2013 we completed subject recruitment and data collection of 285 participants for a total of 414 participants of the 480 participants we had proposed. We partially completed investigation tasks 2 through 5 by quantitating mammographic breast density measurement; recruiting subjects and collecting data; assessing health literacy; and processing blood samples, taking body measurements and performing assays. We partially completed investigation tasks 7 and 8 by conducting ongoing quality assurance audits to ensure patient safety and integrity, and conducting interim analyses.

During the fourth year of the project we continued in the investigation phase. Dr. Khoder left MMC and was replaced by Dr. Nia Foderingham, a Preventive Medicine physician with a Master's of Science in Public Health. Dr. Sanderson attended the Society for Epidemiologic Research conference, and Drs. O'Hara and Foderingham attended the American College of Preventive Medicine conference. The MMC (Drs. Sanderson, O'Hara, Foderingham) and VUMC (Drs. Dupont, Shu, Peterson) investigators presented a poster at the Society for Epidemiologic Research conference and submitted a manuscript for publication. We obtained continuing IRB approval for the project from MMC on 9/8/2014, VUMC on 1/27/2014, and DOD on 9/11/2014. Between October 12, 2013 and March 31, 2014 we completed subject

recruitment and data collection of 62 participants for a total of 476 participants of the 480 participants we had proposed. We fully completed investigation tasks 2 through 5 by quantitating mammographic breast density measurement; recruiting subjects and collecting data; assessing health literacy; and processing blood samples, taking body measurements and performing assays. We fully completed investigation tasks 7 and 8 by conducting ongoing quality assurance audits to ensure patient safety and integrity, and conducting interim analyses.

During the fifth year of the project we continued in the investigation phase. Dr. Foderingham left MMC in March 2015 and was not replaced. Dr. Sanderson attended the AACR Cancer Health Disparities conference, and Drs. O'Hara and Foderingham attended the American College of Preventive Medicine conference. We fully completed investigation task 5 by performing leptin and adiponectin analyses, and investigation task 9 by conducting final analyses and disseminating results to researchers as a published manuscript (Appendix) and to participants as a newsletter (Appendix). Results of our diabetes and mammographic breast density manuscript indicated that after adjustment for confounding variables, the mean percent breast density among premenopausal women with type 2 diabetes ($\hat{\mu}$ 13.8%, 95% confidence interval [CI] 11.6-15.9) was non-significantly lower than that of women without type 2 diabetes ($\hat{\mu}$ 15.9%, 95% CI 15.0-16.8) (p=0.07); however, there was no association among postmenopausal women. The effect of type 2 diabetes in severely obese women (BMI≥35) appeared to differ by menopausal status with a reduction in mean percent breast density in premenopausal women, but an increase in mean percent breast density in postmenopausal women which could have been due to chance. These findings are presented on the following pages. In addition, Drs. Sanderson and Dupont collaborated with former PI, Dr. Fair, on a manuscript from her study of mammographic breast density (Appendix). Dr. Sanderson presented results at the MMC Center for Women's Health Research Grand Rounds in June 2015. We obtained continuing IRB approval for the project from MMC on 6/17/2015, VUMC on 12/12/2014, and DOD on 9/25/2015.

Type 2 Diabetes and Mammographic Breast Density among Underserved Women

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Abstract

Purpose: We conducted a study of women recruited at Meharry Medical College, a Historically Black Medical School, to investigate the relationship between diabetes and mammographic breast density.
Methods: A total of 476 women completed in-person interviews, body measurements and full-field digital mammograms on a Hologic mammography unit from December 2011 through February 2014. Average percent breast density for the left and right breasts combined was estimated using Quantra, an automated algorithm for volumetric assessment of breast tissue. The prevalence of type 2 diabetes was determined by self-report.
Results: After adjustment for confounding variables, the mean percent breast density among premenopausal women with type 2 diabetes ($\hat{\mu}$ 13.8%, 95% confidence interval [CI] 11.6-15.9) was non-significantly lower than that of women without type 2 diabetes ($\hat{\mu}$ 15.9%, 95% CI 15.0-16.8) (p=0.07); however, there was no association among postmenopausal women. The effect of type 2 diabetes in severely obese women (BMI≥35) appeared to differ by menopausal status with a reduction in mean percent breast density in premenopausal women, but an increase in mean percent breast density in postmenopausal women which could have been due to chance.
Conclusions: Confirmation of our findings in larger studies may assist in clarifying the role of the insulin signaling breast cancer pathway in women with high breast density.

Keywords: mammographic breast density, type 2 diabetes, cross-sectional study, underserved

Introduction

Type 2 diabetes has been identified as a weak risk factor for breast cancer, independent of obesity. Meta-analyses of the association between diabetes and breast cancer, consisting primarily of cohort studies, have reported summary relative risks (RR) of approximately 1.20, with 95% confidence intervals (CI) ranging from 1.12 to 1.30 [1-4]. Three of the four meta-analyses stratified by menopausal status at breast cancer diagnosis reported an increased risk of postmenopausal breast cancer associated with diabetes among women, but not among premenopausal women [2-4]. The increase in postmenopausal breast cancer risk associated with diabetes was also reported in a recent large cohort study conducted since these meta-analyses [5]. In another more recent large cohort study, Bowker and colleagues [6] reported that risk for breast cancer diagnosed among women at age 55 years or older, and presumably postmenopausal, was non-significantly increased for 0 to 3 months following diabetes diagnosis (hazard ratio [HR] 1.31, 95% CI 0.92-1.86), but then returned to baseline from 3 months to 10 years following diabetes diagnosis (HR 1.00, 95% CI 0.90-1.11). The authors concluded that the initially elevated postmenopausal breast cancer risk may have been due to detection bias.

High mammographic breast density is a well-established risk factor for breast cancer. Depending on how high mammographic breast density is defined, the range of RRs for breast cancer is around 4 to 6 [7]. In a meta-analysis of 42 studies, the group of women whose fibroglandular tissue comprised ≥75% of breast tissue had a summary RR for breast cancer of 4.64 (95% CI 3.64-5.91) relative to women with <5% [8]. Several breast cancer risk factors that affect the growth (proliferation and apoptosis) and/or differentiation of breast tissue, such as parity, menopause, hormone replacement therapy, body mass index, and hormone levels, are also associated with mammographic breast density [7, 9-10]. Few studies have assessed breast density among Black or Hispanic women. In comparison to White women, Black women have been reported to have denser breasts [11-13], breasts of similar density [14-15] or less dense breasts [16], while Hispanic women have been reported to have breasts of similar density [11, 16]. These studies varied in regard to the age of the study subjects and the methods used to assess breast density.

Although mammographic breast density is thought to be an intermediate phenotype of breast cancer [17], very few studies have investigated the association between diabetes and mammographic breast density. Diabetes may play a role in mammographic breast density through the insulin signaling pathway given that insulin has been linked with low breast density in premenopausal women [18-19]. We conducted a study of women recruited at Meharry Medical College, a Historically Black Medical School, to investigate the relationship between diabetes and mammographic breast density.

Materials and Methods

Study Design

We conducted a clinic-based cross-sectional study of underserved women aged 40 to 79 years recruited at Meharry Medical College, a Historically Black Medical School, between December 2011 and February 2014 to investigate mammographic breast density and its relation with potential breast cancer pathways including insulin [18-19], insulin-like growth factor [10, 20] and adipocytokine [19, 21]. The present study focuses on the insulin signaling pathway by investigating the association between Type 2 diabetes and mammographic breast density. Subjects were eligible if they were underserved meaning their primary place of health care was a safety net clinic. Women were recruited by placing flyers around the campus, and at health fairs and local community agencies. The flyer described the study and asked women to provide contact information if they were interested in participating. Project staff telephoned each woman to evaluate eligibility and to schedule a study appointment. The Institutional Review Boards of Meharry Medical College and Vanderbilt University approved this study's protocol. After informed consent was obtained, women provided a fasting blood sample, underwent body measurements (height, weight, waist, hips, percent body fat) and a digital screening mammogram, and completed an in-person interview on demographics, lifestyle factors, personal health history, family history of cancer and other chronic diseases, adult weight history, diet, and health literacy. Body mass index (BMI) (kg/m^2) and waist-to-hip ratio (WHR) were calculated from body measurements and percent body fat was estimated from a body fat monitor scale.

Study Population

Women who were pregnant, unable to comprehend study materials, or had a history of cancer, breast augmentation or reduction, symptoms of a breast disorder, or a focal dominant lump were ineligible. Premenopausal women were asked the date of their last menstrual period so their appointment could be scheduled during the follicular phase (1-14 days) of their menstrual cycle when their breast tissue is less dense. The day prior to their appointment women were telephoned and reminded to observe a 10-hour fast for their blood draw the following morning. Of the 479 women recruited, exclusions due to incomplete interviews (n=4), type 1 diabetes (n=11), and unknown age at diabetes diagnosis (n=1) resulted in 175 premenopausal women and 288 postmenopausal women for analysis.

Assessment of Breast Density

A trained radiologic technician completed full-field digital screening mammograms on a Hologic mammography unit that uses selenium direct capture technology to eliminate light diffusion completely for perfect clarity and image quality. Our study radiologist (ACD) estimated average percent breast density, defined as the ratio of estimated fibroglandular tissue volume to total breast volume, for the left and right breast combined using Quantra software and assigned Breast Imaging Reporting and Data System (BI-RADS) categories of 0 through 4 represented by increasing density [22]. Subjects with abnormal mammograms (BI-RADS=0: additional imaging evaluation, n=45; BI-RADS=3: probably benign finding, n=3; BI-RADS=4: suspicious abnormality, n= 2) were notified immediately by certified mail, while subjects with normal mammograms were notified of their results within 30 days.

Assessment of Type 2 Diabetes Status

To define diabetes, we used self-reported diabetes from the questionnaire. Women were considered diabetic if they responded "Yes" to the question "Did a doctor or other health care provider ever tell you that you had diabetes, or high sugar in your blood or urine?" on the questionnaire. Women who indicated they had diabetes "Only during pregnancy" on the questionnaire were categorized as non-diabetic. On the questionnaire, women who reported they

had diabetes were then asked how old they were when they were first told they had diabetes and whether they used pills or insulin injections to control their diabetes. Women who indicated their age at diabetes diagnosis was ≤30 years were considered to have type 1 diabetes and were excluded from analysis [23]. For the medication analysis, women who used pills and then switched to insulin to control diabetes were classified as having used insulin.

We conducted a validation study of self-reported diabetes using c-peptide (a biomarker of insulin secretion) which was measured in fasting serum samples using cheminluminescence technology-based assay kits on a proprietary automated moderate complexity endocrine panel (Immulite 1000) according to the manufacturer's instructions (Siemens, Dallas, TX). The calculated sensitivity of the assay (N = 6) was 0.03 ng/tube and the intra-assay coefficients of variation (CVs) for levels 1, 2 and 3 controls (N = 10/level of control) were 2.2%, 3.4% and 3.0%, respectively, within the range of acceptable sensitivity and CVs [24]. The inter-assay CVs were not available because the sera were batch analyzed in two assays. For the validation study, women were considered to have diabetes if their fasting serum c-peptide was >2.0 ng/mL [25].

Statistical Analysis

Statistical analyses were performed in SAS version 9.2. Linear regression was used to estimate mean percent breast density by type 2 diabetes status, while adjusting for confounding variables [26]. We stratified by menopausal status a priori, since fibroglandular breast tissue decreases during the menopausal transition [27]. Interaction terms, the product of diabetes and race/ethnicity (White, Black, Hispanic) and BMI (<35, ≥35), were added to linear regression models and likelihood ratio tests were performed to test for effect measure modification. Covariates examined as potential confounders of the relationship between diabetes and mean percent breast density included race/ethnicity, age, education, family history of breast cancer, family history of diabetes, age at menarche, parity, age at first pregnancy, oral contraceptive use, smoking, alcohol intake, physical activity, BMI, WHR, percent body fat, age at menopause and hormone replacement therapy (HRT) use. Confounders were examined as categorized in Table 1 with the exception of age, BMI, WHR and percent body fat which were examined continuously.

Variables were considered confounders if their addition to the model changed the unadjusted mean percent breast density by 10 percent or more. There was no evidence of statistical interaction for race/ethnicity or BMI; however, we present results for type 2 diabetes stratified by BMI since the effect of diabetes on mean percent breast density appears to be most pronounced among severely obese women. In addition, we stratified by menopausal status and adjusted for race/ethnicity, age and BMI (as needed), and additionally for HRT use among postmenopausal women which met our criteria for model inclusion. Adjustment for WHR and percent body fat did not meet our criteria for confounding. For our validation study of self-reported diabetes, we used serum c-peptide as the gold standard and calculated sensitivities and specificities and their respective confidence intervals as measures of validity. Lastly, we performed a sensitivity analysis by examining our findings with and without the inclusion of 50 women with abnormal mammograms and our results were similar.

Results

Table 1 presents the demographic characteristics and breast cancer risk factors of participants by menopausal status. In both premenopausal and postmenopausal groups, we observed a high prevalence of several breast cancer risk factors including family history of breast cancer, younger age at menarche, alcohol intake, no physical activity and high body measurements. The percentage of all women reporting a family history of diabetes was extremely high (premenopausal 62.3%; postmenopausal 69.9%).

Table 2 presents mean percent breast density associated with type 2 diabetes by menopausal status. After adjustment for confounding variables, the mean percent breast density among premenopausal women with type 2 diabetes ($\hat{\mu}$ 13.8%, 95% confidence interval [CI] 11.6-15.9) was non-significantly lower than that of women without type 2 diabetes ($\hat{\mu}$ 15.9%, 95% CI 15.0-16.8) (p=0.07); however, there was no association among postmenopausal women. Among severely obese (BMI≥35) premenopausal women, the mean percent breast density was non-significantly lower in women with diabetes ($\hat{\mu}$ 10.8%, 95% CI 8.3-13.2) than in women without diabetes ($\hat{\mu}$ 13.1%, 95% CI 11.9-14.4) (p=0.07). However, the opposite was true in severely obese postmenopausal women with a significantly higher mean percent breast density in women with diabetes ($\hat{\mu}$ 12.8%, 95% CI 11.8-13.8) than in women without diabetes ($\hat{\mu}$ 11.1%, 95% CI 10.1-12.0) (p=0.01). While premenopausal women whose diabetes was diagnosed at least 10 years ago had lower mean percent breast density than women diagnosed less than 5 years ago, the opposite was true for postmenopausal women. There was no effect of the use of insulin or pills among diabetics on mean percent breast density.

To ascertain misclassification of self-reported diabetes we conducted a validation study using fasting serum c-peptide available for 95% of subjects as the gold standard. Results indicated very low sensitivity (22.8, 95% CI 18.0-28.3) and high specificity (83.2, 95% CI 77.2-87.9) of self-report of diabetes in comparison with serum c-peptide. The total percentage of women whose c-peptide level indicated diabetes (58.0%) was 37.8% higher than the percentage of women who self-reported diabetes (20.2%). This percentage is higher than the estimated 27.8% of undiagnosed diabetes in the U.S. [28], but may be due to the high rates of obesity (BMI 30-34.9; 25%) and severe obesity (BMI≥35; 29%) in our study population.

Discussion

We found a non-significantly lower mean percent density associated with self-reported diabetes among premenopausal women, but no association in postmenopausal women after continuous adjustment for BMI. This finding is in agreement with two studies of self-reported diabetes and mammographic breast density. Robidoux et al. [18], in a study of Southwestern Native-American women, classified breast density using BI-RADS categories analog mammograms, and found that self-reported diabetes was associated with lower breast density (moving up from one BI-RADS category to the next) in premenopausal ($p=0.0032$) but not in postmenopausal women ($p=0.3178$). Sellers et al. [29], in a study of primarily postmenopausal White women in Minnesota, found no association between self-reported type 2 diabetes and breast density based on a computer-assisted thresholding program (Cumulus) [30] of analog mammograms in premenopausal or postmenopausal women. However, these investigators did identify a positive association between diabetes and breast cancer.

This finding is in partial agreement with two other studies that investigated c-peptide levels and breast density which found overall or within strata of menopausal status [31-32]. Diorio et al. [31], in a study of primarily White women in Quebec City, found no association between non-fasting c-peptide levels and breast density based on the Cumulus thresholding program after adjustment for BMI and WHR ($p=0.41$), and after stratification by menopausal status ($p=0.94$). Ahern et al. [32], in a case-control study nested within the Nurses' Health Study cohorts, found no association between fasting c-peptide levels and breast density measured using Cumulus after adjustment for BMI and WHR in premenopausal and postmenopausal women combined and within starta of menopausal status. As was the case with Sellers et al. [29], these investigators did identify a positive association between diabetes and breast cancer.

Among self-reported diabetics in our study, premenopausal women whose diabetes was longer standing had lower mean percent breast density than women diagnosed more recently. To our knowledge, no other study has investigated breast density as it relates to the time since diabetes diagnosis. Our failure to find an effect of diabetes treatment on breast density may have been due to limited statistical power, but was unexpected given the recent interest in utilizing metformin, one of the most common oral diabetes medications, as a breast cancer chemopreventive agent [33], particularly in postmenopausal women [34]. To date, one study has investigated the effect of metformin and breast density in postmenopausal women and reported a decrease in 7 of 14 women after 10.5 months of use that was more pronounced in women with no signs of metabolic syndrome [35].

Our study was potentially limited by selection bias since our sample was one of convenience. In addition, statistical power was limited, especially when we stratified by both menopausal status and BMI, so these results should be interpreted with caution. Also misclassification of breast density could have affected our results since we used Quantra, a fairly new automated algorithm for volumetric assessment of breast tissue, rather than the standard computer-assisted Cumulus thresholding program. To date, the validity of Quantra has yet to be established. In comparing Quantra with magnetic resonance imagining (MRI), Wang et al. [36] reported lower median percent breast density with Quantra (22.0%, interquartile range [IQR] 14.0%) than with MRI (24.0%, IQR 36.0%). Ciatto et al [37] reported systematically lower percent breast density with Quantra compared with visual classification using BI-RADS by eleven experienced radiologists, but the authors maintained that its reproducibility makes it preferable to visual classification. Engelken et al. [38] reported a Pearson correlation coefficient of 0.920 ($p<0.05$) for serial digital mammograms using Quantra software on the same unit within a 24-month period.

Very few epidemiologic studies of breast density have utilized full-field digital mammograms with Quantra software for comparison with our study. The mean breast density (19.7%, range 8.5%-48.5%) and age (59 years, range 49-81 years) of an English study of premenopausal and postmenopausal women combined [39] were higher than that of our study (breast density 14.1%, range 6.5%-34.0%; age 51 years, range 40-76 years). In a German study, Hammann-Kloss et al. [40] reported median breast densities for women of <46 years (28%, IQR 15.0%), 46-55 years (23.0%, IQR 15.3%) and >55 years (16.0%, IQR 10.0%) that were higher than those of our study (<46 years 15.0%, IQR 8.25%; 46-55 years 12.5%, IQR 5.5%; >55 years 11.5%, IQR 3.75%). Both of these findings may have been due to chance or due to the high prevalence of obesity, and therefore less dense breasts, in our population.

Strengths of our study included the high rates of diabetes in our population, a priori stratification by menopausal status, adjustment for known confounders, the validation study of self-reported diabetes, and the examination of findings with and without women who had abnormal mammograms. To our knowledge, our study is the first to identify a suggested association between type 2 diabetes and mammographic breast density in severely obese women that appeared to operate in opposite directions in premenopausal and postmenopausal women which could have been due to chance. Most studies of diabetes and breast density have only identified weak associations in premenopausal women suggesting that the link between diabetes and breast cancer is not mediated through breast density. Confirmation of our findings in larger studies may assist in clarifying the role of the insulin signaling breast cancer pathway in women with high breast density and ultimately target those women who will benefit most from primary and secondary prevention.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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Table 1. Demographic characteristics and breast cancer risk factors of participants by menopausal status

	Premenopausal (n=175)		Postmenopausal (n=288)	
Characteristic	n	%	n	%
Race				
White	36	20.6	74	25.7
Black	79	45.1	172	59.7
Hispanic	60	34.3	42	14.6
Age (years)				
40-49	157	89.7	59	20.5
50-64	18	10.3	178	61.8
65-79	0	0.0	51	17.7
Education				
< High school	49	28.3	65	22.6
High school graduate	41	23.7	74	25.7
Some college	55	31.8	91	31.6
College graduate	28	16.2	58	20.1
Missing	2		0	
Family history of breast cancer				
No	116	66.3	184	64.8
Yes	50	28.5	91	32.0
Adopted	8	4.6	8	2.8
Don't know	1	0.6	1	0.4
Missing	0		4	
Family history of diabetes				
No	58	33.1	77	26.9
Yes	109	62.3	200	69.9
Adopted	8	4.6	8	2.8
Don't know	0	0.0	1	0.4
Missing	0		2	
Age at menarche (years)				
≤12	85	48.6	143	49.7
13	35	20.0	68	23.6
>13	55	31.4	77	26.7
Number of full-term pregnancies				
0	23	13.2	23	8.0
1-2	43	24.7	90	31.4
3-4	72	41.4	109	38.0
≥5	36	20.7	65	22.6
Missing	1		1	
Age at first pregnancy (years)[a]				
<30	134	89.3	247	95.0
≥30	16	10.7	13	5.0
Missing	1		4	
Oral contraceptive use				
No	50	28.9	77	26.7
Yes	123	71.1	211	73.3
Missing	2		0	

Table 1. Demographic characteristics and breast cancer risk factors of participants by menopausal status

	Premenopausal (n=175)		Postmenopausal (n=288)	
Characteristic	n	%	n	%
Smoking				
No	104	59.4	116	40.4
Yes	71	40.6	171	59.6
Missing	0		1	
Alcohol intake				
No	99	56.9	135	47.2
Yes	75	43.1	151	52.8
Missing	1		2	
Physical activity				
None	54	30.9	97	33.8
Moderate	63	36.0	116	40.4
Strenuous	58	33.1	74	25.8
Missing	0		1	
Body mass index				
<25	25	14.4	52	18.1
25-29.9	56	32.4	77	26.8
30-34.9	41	23.7	75	26.2
≥35	51	29.5	83	28.9
Missing	2		1	
Waist-to-hip ratio				
<0.84	49	28.3	66	23.0
0.84-0.88	40	23.1	75	26.1
0.89-0.92	51	29.5	64	22.3
≥0.93	33	19.1	82	28.6
Missing	2		1	
% Body fat				
<37.9	45	26.5	64	22.6
37.9-43.0	51	30.0	67	23.7
43.1-47.2	34	20.0	78	27.6
≥47.3	40	23.5	74	26.1
Missing	5		5	
Age at menopause (years)[b]				
<50			218	75.7
50-54			54	18.7
≥55			12	4.2
Don't know			4	1.4
Hormone replacement therapy use[b]				
No			199	69.3
Yes			88	30.7
Missing			1	

[a]Among parous.
[b]Among postmenopausal.

Table 2. Mean percent breast density associated with type 2 diabetes by menopausal status

Characteristic	Premenopausal				Postmenopausal			
	n	Mean % density[a]	95% CI	P-value	n	Mean % density[b]	95% CI	P-value
Type 2 diabetes								
Overall								
No	151	15.9	15.0-16.8		221	13.0	12.3-13.6	
Yes	24	13.8	11.6-15.9	0.07	67	13.1	12.1-14.2	0.78
BMI<35								
No	108	17.0	15.8-18.2		167	13.5	12.7-14.3	
Yes	14	15.1	11.9-18.2	0.25	37	13.0	11.5-14.5	0.49
BMI≥35								
No	41	13.1	11.9-14.4		54	11.1	10.1-12.0	
Yes	10	10.8	8.3-13.2	0.07	29	12.8	11.8-13.8	0.01
Times since diabetes diagnosis (years)[b]								
<5	16	14.2	12.4-16.0	Referent	31	12.3	10.9-13.8	Referent
5-9	4	10.5	6.7-14.3	0.07	17	11.7	9.8-13.6	0.55
≥10	4	10.2	6.4-14.0	0.05	19	14.8	13.0-16.5	0.03
Diabetes medications[b]								
None	6	13.9	11.0-16.9	Referent	12	13.4	11.2-15.6	Referent
Insulin	11	13.7	11.2-16.2	0.90	30	12.3	10.8-13.9	0.42
Pills	7	10.9	7.6-14.2	0.16	25	13.3	11.5-15.1	0.94

[a]Adjusted for race/ethnicity, age, and BMI (as needed).
[b]Adjusted for race/ethnicity, age, BMI (as needed) and HRT use.
[c]Among self-reported diabetics.

Key Research Accomplishments

- Fully completed training tasks 1a through 1l by Drs. Sanderson, Jones/O'Hara and Khoder/Foderingham attending and/or presenting posters at workshops and conferences, Dr. Jones taking coursework in the MSCI Program, consulting with our advisory board and health providers in the MMC Center for Women's Health Research to design the breast density study, developing study protocols, posters, informed consent documents, standard operating procedures, questionnaires and databases, and by obtaining IRB approval from three entities.

- Fully completed investigation tasks 2 through 5 by recruiting subjects and collecting and processing data (digital mammograms, blood, body measurements, questionnaires including health literacy).

- Fully completed investigation tasks 7 through 9 by conducting quality assurance audits and interim and final analyses, and disseminating results to researchers and participants.

Reportable Outcomes

1) Manuscripts

Sanderson M, Perez A, Weriwoh ML, Alexander LR, Peltz G, Agboto V, O'Hara H, Khoder W. Perinatal factors and breast cancer risk among Hispanics. J Epidemiol Global Health 2013;3:89-94.

Sanderson M, O'Hara H, Foderingham N, Dupont WD, Shu X-O, Peterson N, Fair AM, Disher AC. Type 2 diabetes and mammographic breast density among underserved women. Cancer Causes Control 2015; 26:303-309.

Fair AM, Lewis TJ, Sanderson M, Dupont WD, Fletcher S, Egan KM, Disher AC. Increased vitamin D and calcium intake associated with reduced mammographic breast density among premenopausal women. Nutr Res 2015; 35:851-857.

2) Abstracts

Sanderson M, Fair AM, Jones C, Khoder W, Dupont W, Shu XO, Peterson N. Mammographic breast density in a cohort of medically underserved women. 6th Department of Defense Breast Cancer Research Program Era of Hope Meeting, Orlando, FL, August 2011.

Sanderson M, Weriwoh M, Peltz, Perez A, Johnson M, Fadden MK. Perinatal factors and breast cancer risk among Latinas. 6th Department of Defense Breast Cancer Research Program Era of Hope Meeting, Orlando, FL, August 2011.

Jones CD, Pryor JL. A combination of marketing and information technology to grow community awareness and to expedite translational research projects. 4th Annual National CTSA Community Engagement Conference, Bethesda, MD, August 2011.

Sanderson M, Perez A, Weriwoh ML, Alexander L, Peltz G, Agboto V, Jones CD, Khoder W. Perinatal factors and breast cancer risk among Hispanics. Am J Epidemiol 2012;175:S6.

Sanderson M, Bevel MS, Alexander L, Fair AM, Peltz G, O'Hara, Khoder W. Hormone replacement therapy and breast cancer among Hispanics. 13th RCMI International Symposium on Health Disparities. San Juan, Puerto Rico, December 2012.

Sanderson M, O'Hara H, Foderingham N, Dupont WD, Shu X-O, Peterson N, Fair AM, Fadden MK. Diabetes and mammographic breast density among white and black women. Am J Epidemiol 2014;179:L02.

Sanderson M. The impact of diabetes on breast density and breast cancer. MMC Center for Women's Health Research Grand Rounds, June 2015.

3) Lay version of results for participants

A lay version of the results including the demographic characteristics of participants by menopausal status and the diabetes and breast density manuscript was completed and mailed to all participants.

4) Grants

Not applicable

Conclusions

The overall goal of this HBCU/MI Partnership Training Award was to strengthen the existing collaborative relationship between the minority institution, MMC, and the collaborating institution, VUMC. The investigators from MMC and VUMC have mutual interests in studying the interplay of lifestyle and molecular factors on breast cancer risk as measured by its precursor, mammographic breast density. High mammographic breast density is comparable in its predictive magnitude of risk to historically well-established breast cancer risk factors. The biological basis for the association between higher percentage of density and risk of breast cancer is not clear but may be related to increased stroma and glandular tissue in dense breasts through estrogen exposures or production of certain growth factors including insulin-like growth factor-I (IGF-I) or adipokines such as leptin. Very few studies have focused on obesity and insulin resistance as they relate to mammographic breast density. We hypothesized that: 1) obesity and insulin resistance, defined as high levels of C-peptide, would be positively associated with high mammographic breast density, and 2) these associations would be more pronounced among women with high levels of IGF-I and high levels of leptin.

This project will establish associations between some lifestyle and molecular factors and mammographic breast density; known to be linked to subsequent breast cancer, especially in minority and medically underserved women. By identifying biomarkers that influence mammographic breast density in minority women, this project may provide therapeutic targets for new prevention strategies in this population. While faculty from VUMC has expertise in breast cancer research, faculty from MMC has strong ties with minority communities in Nashville and Davidson County. To date, limited breast cancer research has been conducted at MMC. By partnering together, MMC and VUMC hope to build infrastructure to conduct

population-based case-control studies of breast cancer at MMC, and to establish an outstanding collaborative breast cancer research program.

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Sanderson M, Perez A, Weriwoh ML, Alexander LR, Peltz G, Agboto V, O'Hara H, Khoder W. Perinatal factors and breast cancer risk among Hispanics. J Epidemiol Global Health 2013;3:89-94.

Sanderson M, O'Hara H, Foderingham N, Dupont WD, Shu X-O, Peterson N, Fair AM, Disher AC. Type 2 diabetes and mammographic breast density among underserved women. Cancer Causes Control 2015; 26:303-309.

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Sanderson M, Weriwoh M, Peltz, Perez A, Johnson M, Fadden MK. Perinatal factors and breast cancer risk among Latinas. 6th Department of Defense Breast Cancer Research Program Era of Hope Meeting, Orlando, FL, August 2011.

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Sanderson M, Bevel MS, Alexander L, Fair AM, Peltz G, O'Hara, Khoder W. Hormone replacement therapy and breast cancer among Hispanics. 13th RCMI International Symposium on Health Disparities. San Juan, Puerto Rico, December 2012.

Sanderson M, O'Hara H, Foderingham N, Dupont WD, Shu X-O, Peterson N, Fair AM, Fadden MK. Diabetes and mammographic breast density among white and black women. Am J Epidemiol 2014;179:L02.

Sanderson M. The impact of diabetes on breast density and breast cancer. MMC Center for Women's Health Research Grand Rounds, June 2015.

Personnel

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Statement of Work

Phase 1: Training Phase (Year 1)

Task 1: (Drs. Sanderson, Khoder, Jones, Richard-Davis, Disher, Sanderson, Dupont, Peterson and Shu) (Jones replaced by O'Hara and Khoder replaced by Foderingham)

1a. Drs. Sanderson, Khoder and Jones audit courses at Summer Research program at University of Michigan (months 6-7).
1b. Dr. Jones begins the Meharry Medical College, Master's of Science in Clinical Investigation Program (months 1-30).
1c. Consult with advisory board and health providers in the Center for Women's Health Research (CWHR) to design a cross-sectional study for measurement of mammographic breast density, related hormones and health literacy (months 1-3).
1d. Develop and finalize study protocol for recruitment of participants (months 1-6).
1e. Develop and finalize study protocol for obtaining analog screening mammograms and digital mammograms (months 1-3).
1f. Finalize advertisements for contacting participants, questionnaires, and other data collection forms (months 1-3).
1g. Order supplies for blood collection and processing, order supplies for performing assays (months 5-6).
1h. Create and finalize quality assurance audit forms to ensure safety of participants and integrity of all data (months 4-6).
1i. Update IRB protocols, informed consent documents, and HIPAA waivers for IRB submission (months 4-6).
1j. Generate standard operating procedures manual to reflect all aspects of study procedures (months 4-6).
1k. Work with Dr. Dupont to modify accrual database to include scripts and screening forms, and allow accrual and productivity reports to be generated (months 7-12).
1l. Work with the project coordinator to create REDCAP database for entry of study data (months 7-12).

Phase 2**:** Investigation Phase (Years 1 through 5)

Specific Aim 1) to assess mammographic breast density through digital mammograms; for a sample of women we will also assess mammographic breast density through analog mammograms to determine the efficacy of digital versus analog mammogram;

Specific Aim 2) to obtain information on breast cancer risk factors including health literacy, and to collect anthropometric measurements and fasting blood;

Specific Aim 3) to assay blood for select hormones and growth factors;

Specific Aim 4) to perform statistical analyses to determine the association between obesity and insulin resistance and mammographic breast density;

Specific Aim 5) to evaluate patients' ability to understand their mammogram findings as they are explained by their medical provider.

Task 2: (Drs. Sanderson, Dupont, Disher, Khoder) (Khoder replaced by Foderingham)
Quantitate mammographic breast density measurement, Months 1-42.

2a. Work with Dr. Disher to refine protocols for mammographic density analyses (months 1-12).
2b. Work with Dr. Disher to observe Cumulus computer program to quantify breast density (months 7-12).
2c. Coordinate flow of digital mammography data from the Center of Women's Health Research to Dr. Disher for quantitation (months 7-42).
2d. Assess breast density of mammograms using digital quantitative analysis to obtain the percentage of the breast occupied by breast tissue (months 7-42).

Task 3: (Drs. Sanderson, Jones, Disher) (Jones replaced by O'Hara)
Recruit subjects and collect data, Months 7-42.

3a. Screen and recruit potentially eligible women for digital mammography study at the Center for Women's Health Research (1,000 patients total) (months 7-42).
3b. Administer questionnaire (months 7-42).
3c. Perform standardized body measures; weight, height, skinfold thickness, and waist and hip circumference (months 7-42).
3d. Collect blood samples and transport to Vanderbilt molecular epidemiology laboratory for storage and processing (months 7-42).
3e. Order additional supplies as needed (months 7-42).

Task 4: (Drs. Jones, Khoder and Peterson**)** (Jones replaced by O'Hara and Khoder replaced by Foderingham) Months 7-42.

4a. Administer Short Test of Functional Literacy in Adults **(**S-TOFHLA) to study participants (months 7-42).
4b. Score S-TOFHLA instruments and categorize levels of patient's health literacy (months 7-42).

Task 5: (Drs. Sanderson, Jones, Khoder and Shu) (Jones replaced by O'Hara and Khoder replaced by Foderingham)
Process blood samples, measurements and perform stated assays, Months 7-42.

5a. Supervise research staff in acquisition and analysis of data (months 7-42).
5b. Separate serum, plasma and clot in blood sample and store at -80°C (months 7-42).
5c. Transport biospecimens to the Vanderbilt University molecular epidemiology laboratory for processing and analysis (months 7-42).

Task 6: (Drs. Khoder, Disher and Dupont) (Khoder replaced by Foderingham) Months 7-42.

6a. Obtain analog mammography films and digital mammography films for each participating patient for rating of quantitative breast density by interpretation (months 7-42).
6b. Calculate the sensitivity and specificity of each modality for detecting mammographic breast density (months 7-42).
6c. Perform statistical analyses to account for multiple comparisons in breast density subgroups (months 40-42).

Task 7: (Drs. Sanderson, Jones, Khoder **,** Dupont) (Jones replaced by O'Hara and Khoder replaced by Foderingham)

Conduct ongoing quality assurance audits to ensure patient safety and data integrity, Months 7-48. Twice monthly monitoring of activities (number of screening phone calls logged, number and type of contacts with potential or actual participants, progress with data entry, etc.).

7a. Twice monthly monitoring of study accrual (months 7-42).
7b. Continuous monitoring/reporting of potential adverse events (months 7-48).
7c. Monthly audits to verify study staff adherence to standard operating procedures (months 7-48).

Task 8: (Drs. Sanderson, Jones, Khoder, Shu, Dupont, Peterson) (Jones replaced by O'Hara and Khoder replaced by Foderingham)

Conduct interim analyses, Months 12-48.

8a. Perform interim statistical analysis (months 12-18, months 24-30, months 36-42).
8b. Preparation and submission of abstracts reflecting findings to date (months 36-48).
8c. Creation and submission of annual reports to funding agency (months 12, 24, 36).

Task 9: (Drs. Sanderson, O'Hara, Khoder, Shu, Dupont, Peterson)

Final analyses and dissemination of data, Months 40-58.

9a. Begin final statistical analyses (months 40-58).
9b. Preparation and submission of final report to funding agency (months 58).
9c. Preparation and submission of abstracts and manuscripts reflecting final results (months 40-58).

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Perinatal factors and breast cancer risk among Hispanics

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KEYWORDS
Breast neoplasms;
Prenatal exposure delayed effects;
Risk factors;
Hispanic Americans;
Case–control studies

Abstract *Purpose:* This study assessed whether perinatal factors were associated with breast cancer among Hispanics, a group with fairly low incidence rates of breast cancer.

Methods: Data were used from a case–control study of breast cancer among Hispanics aged 30–79 conducted between 2003 and 2008 on the Texas–Mexico border. In-person interviews were completed with 188 incident breast cancer cases ascertained through surgeons and oncologists, and 974 controls (with respective response rates of 97% and 78%).

Results: Relative to birth weight 2500–3999 g, there was no elevation in breast cancer risk for birth weight of $\geqslant$4000 g (odds ratio [OR] 0.76, 95% confidence interval [CI] 0.47–1.21).

Conclusions: The results tended to differ slightly from previous studies of this topic perhaps owing to the different hormonal milieu among Hispanics relative to Caucasians, African Americans and Asians in whom all previous studies of this topic

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have been conducted. Confirmation of these findings in larger studies may assist in determining how hormonal mechanisms responsible for breast cancer differ by ethnicity.

1. Introduction

High birth weight and other perinatal factors thought to reflect on a woman's exposure to hormones, growth factors and other endocrine factors have been linked to subsequent breast cancer [1]. Three meta-analyses of the high birth weight-breast cancer association have reported summary relative risks ranging from 1.15 (95% confidence interval [CI] 1.09—1.21) to 1.24 (95% CI 1.04—1.48) [2—4], while a pooled analysis of this association based on birth records reported a pooled relative risk of 1.12 (95% 1.00—1.25) [5]. High birth weight was defined as $\geqslant$4000 g relative to <3000 g for the most part in the meta-analyses [2—4] or relative to 3000—3499 g in the pooled analysis [5]. After restricting the types of studies to cohort studies, two meta-analyses of the association between older maternal age defined as $\geqslant$30 years relative to <25 years and breast cancer reported summary relative risks of 1.13 (95% CI 1.02—1.25) [2] and 0.99 (95% CI 0.82—1.19) [3], respectively. Neither higher birth order (relative risk [RR] 0.91, 95% CI 0.91—1.04) nor maternal smoking (RR 0.98, 95% CI 0.86—1.13) appeared to be associated with breast cancer in a meta-analysis that included studies of all types [3]. Meta-analyses have reported breast cancer to be positively associated with birth length and older paternal age [2], negatively associated with pre-eclampsia/eclampsia and twin membership [2], and not associated with gestational age [2,3], and maternal diethylstilbestrol (DES) use [2]. However, cohort studies have identified a positive association between maternal DES and breast cancer among women diagnosed at age 40 or older [6,7]. None of the studies reported on the meta-analyses or pooled analysis examined the associations between perinatal factors and breast cancer among Hispanic women who have fairly low incidence rates of breast cancer compared with Caucasian women [8].

Based on mothers who delivered between 1974 and 1977, the birth characteristics of Hispanic women also differ from those of Caucasian women [9]. In comparison with Caucasians, Hispanics weigh slightly less (3.48 vs. 3.42 kg), are born to younger mothers (26.5 vs. 25.7 years), are of higher birth order (18.6% $\geqslant$2 vs. 26.0% $\geqslant$2), and are born to mothers who do not smoke during pregnancy (70.1% vs. 79.4%). Given the differences in perinatal factors and breast cancer incidence rates of Hispanics relative to Caucasians, it was assessed whether perinatal factors were associated with breast cancer among Hispanic women in the current study.

2. Materials and methods

Detailed methods of this clinic-based case—control study conducted in the Lower Rio Grande Valley located at the southern tip of Texas on the Mexico border appear elsewhere [10]. Briefly, cases of self-reported Hispanic ethnicity, aged 30—79, diagnosed with primary invasive breast cancer between November 2003 and August 2008 were identified through surgeons and oncologists shortly after diagnosis or treatment (n = 190, response rate 97.0%). Controls of Hispanic ethnicity, aged 30—79, were randomly selected from women receiving a diagnostic or screening mammogram at the mammography center where the case received her diagnostic mammogram. Interviews were completed with approximately five controls per case (n = 979, response rate 78.0%). Women who were adopted were excluded resulting in 188 cases, and 974 controls for analysis.

Written informed consent was obtained from subjects and the Institutional Review Boards of the University of Texas at Brownsville and the University of Texas Health Science Center at Houston approved this study's protocol. Trained interviewers conducted in-person interviews on demographic characteristics, suspected breast cancer risk and protective factors, medical history, physical activity, diet, body size and perinatal factors. Exposures were for a period before a reference date, the date of diagnosis for the cases and an assigned date for controls comparable to the date for the cases. For example, controls recruited early in the study were assigned reference dates ranging from November 2003 to December 2005, while controls recruited later in the study were assigned reference dates ranging from January 2006 to August 2008.

Statistical analyses were completed in SAS version 9.2. There were large percentages of missing

data for some perinatal factors (birth weight 14.2%, maternal age 13.7%, and maternal hormone use 18.1%). It was assumed that these missing values were missing at random and multiple imputation for handling these missing values were implemented. The variables listed in Tables 1 and 2 were used to perform 10 imputations under a multivariate normal model. An assumption of multiple imputation is that all variables are normally distributed which, based on a normal probability plot, was not the case for body mass index (BMI). BMI was log transformed for the imputation models and retransformed for presentation in Table 1. Logistic regression was used to estimate the relative risk of breast cancer associated with perinatal factors while controlling for potential confounding factors [11]. To assess the fit and any influential observations of the logistic regression models, Pregibon's diagnostics measures were implemented, including index plots and delta-betas [12]. Some observations were influential, but their impact on the fit was negligible. Overall, there were no concerns regarding the fitted models. Age, family history of breast cancer, age at menarche, menopausal status, parity, BMI, use of oral contraceptives, use of hormone replacement therapy, alcohol intake, number of mammograms in past 6 years, physical activity and other perinatal factors were evaluated as potential confounders. An alpha level of 0.05 was used to determine statistical significance of all two-sided statistical tests, and final analyses are presented using Rubin's rules for reporting summary statistics, odds ratios, confidence intervals, test statistics and diagnostic measures from the 10 multiple imputations [13].

3. Results

Table 1 presents the distribution of suspected breast cancer risk and protective factors by case—control status following the imputation of missing values. Cases were more likely than controls to be older, to have a family history of breast cancer, to have an earlier age at menarche, to be postmenopausal, not to have used oral contraceptives or hormone replacement therapy, to have had fewer mammograms in the past 6 years, and not to have engaged in physical activity.

The addition of age modeled continuously, menopausal status and number of mammograms in the past 6 years to the perinatal factors-breast cancer models changed the crude odds ratio by 10% or more, so adjustment was made for these confounding variables. There appeared to be no association with breast cancer among women whose birth weight was 4000 g or more relative to women whose birth weight was 2500—3999 g (odds ratio [OR] 0.76, 95% CI 0.47—1.21 after adjustment for age, menopausal status and mammography screening) (Table 2). Nor were women who were born preterm at risk of breast cancer relative to women who were born at term (OR 0.32, 95% CI 0.08—1.40). Although there did appear to be an increased risk odds of breast cancer associated with twin birth (OR 2.83, 95% CI 1.08—7.37) and maternal smoking (OR 1.44, 95% CI 0.85—2.45), the wide confidence intervals argue for cautious interpretation. There was no association with breast cancer risk odds for older maternal age or higher birth order.

4. Discussion

The results of this study, which were not statistically significant and tended to differ only slightly from previous meta-analyses [2—4] and a pooled analysis [5] of this topic, are scientifically interesting. A possible explanation for these results may be the different hormonal milieu among Hispanics relative to Caucasians, African Americans and Asians in whom all previous studies of this topic have been conducted. A recent study in the southwestern United States found that two estrogen-related factors — hormone replacement therapy and younger age at menarche — do not function as risk factors for breast cancer diagnosed after menopause among Hispanic women as they do among Caucasian women [14]. Hines et al. [14] hypothesized that the ethnic differences in postmenopausal breast cancer associated with estrogen exposure may be modified by genetic, environmental and/or lifestyle factors. They speculated this may be reflected in the higher proportion of estrogen receptor positive tumors in Caucasian women than in Hispanic women [15].

Another possible explanation for the different findings from previous studies is that *in utero* exposures may not act directly on the breast, but may alter other physiologic pathways that affect risk later in life. Terry et al. [16] investigated the cohort of daughters whose mothers participated in the New York site of the Collaborative Perinatal Project from 1959 to 1963 and found no differences in age at menarche by birth weight, maternal age, birth order, gestational age, or maternal smoking. Troisi et al. [1] indicated there is insufficient evidence to establish associations between perinatal factors and premenopausal estrogen or adult insulin-like growth factor levels, both thought to be related to breast cancer risk.

Table 1 Comparison of cases and controls for suspected breast cancer risk and protective factors.

Characteristic	Cases (n = 188)		Controls (n = 974)	
	N	%	*N*	%
Age (years)				
30–49	61	32.4	391	40.1
50–64	87	46.3	472	48.5
65–79	40	21.3	111	11.4
Breast cancer among first-degree relatives				
No	168	89.4	905	92.9
Yes	20	10.6	69	7.1
Age at menarche (years)				
<12	50	26.7	228	23.4
⩾13	138	73.3	746	76.6
Menopausal status				
Premenopausal	39	21.0	281	28.8
Postmenopausal	149	79.0	693	71.2
Full-term pregnancy				
No	10	5.3	60	6.2
Yes	178	94.7	914	93.8
Body mass index				
<25	13	7.1	69	7.1
25–29.9	44	23.6	230	23.6
30–34.9	77	41.2	401	41.2
⩾35	54	28.1	274	28.1
Oral contraceptive use				
No	66	35.3	267	27.4
Yes	122	64.7	707	72.6
Hormone replacement therapy use[a]				
No	90	60.3	431	44.3
Yes	59	39.7	543	55.7
Alcohol intake				
No	154	81.9	798	81.9
Yes	34	18.1	176	18.1
Number of mammograms in past 6 years				
0–1	39	20.7	97	10.0
2–3	54	28.7	187	19.2
4–5	34	18.1	186	19.1
⩾6	61	32.4	504	51.7
Physical activity				
No	115	61.2	485	49.8
Yes	73	38.8	489	50.2

[a] Among postmenopausal women.

Lastly, these results may have been explained by insufficient study power. This study power was limited for all main effects; in order to achieve 80% power for the high birth weight-breast cancer association, this study would have required 725 cases and 2900 controls.

This study was limited by self-report of perinatal factors which is prone to misclassification and resulted in many missing values. Several validation studies of perinatal factors have been performed, including one that was conducted on women born in Washington State in which very high correlations comparing self-report with birth certificate for maternal age (r = 0.95), and comparing self-report with mother report for birth order (r = 0.89) and for birth weight (r = 0.85) [17] were found.

Table 2 Odds ratios of breast cancer associated with perinatal factors.

Characteristic	Cases (n = 188)		Controls (n = 974)		OR[a]	(95% CI)
	N	%	N	%		
Birth weight (g)						
<2500	28	15.1	164	16.8	0.76	(0.47–1.21)
2500–3999	146	77.3	708	72.7	1.00	(Referent)
⩾4000	14	7.6	102	10.6	0.68	(0.36–1.29)
Maternal age (years)						
<25	84	44.8	392	40.2	1.00	(Referent)
25–29	42	22.3	226	23.2	0.92	(0.58–1.46)
⩾30	62	32.9	356	36.6	0.84	(0.57–1.25)
Birth order						
First	40	21.1	205	21.0	1.00	(Referent)
⩾Second	148	78.9	769	79.0	1.00	(0.95–1.05)
Gestational age (weeks)						
<37	2	1.3	27	2.8	0.32	(0.08–1.40)
⩾37	186	98.7	947	97.2	1.00	(Referent)
Twin birth						
No	180	95.7	962	98.8	1.00	(Referent)
Yes	8	4.3	12	1.2	2.83	(1.08–7.37)
Maternal smoking						
No	164	87.3	893	91.7	1.00	(Referent)
Yes	24	12.7	81	8.3	1.44	(0.85–2.45)

[a] Odds ratio (OR) and 95% confidence interval (95% CI) adjusted for age, menopausal status and number of mammograms in past 6 years.

The percentage of women unable to report some of their perinatal factors ranged from 1.6% for birth order to 18.1% for maternal hormone use. With the exception of gestational age, cases were slightly more likely than controls to have missing values. Although the percentages of missing values tended to be similar for cases and controls, it was not clear as to whether the missing value would have been systematically lower or higher than the obtained value, thus multiple imputations may have resulted in a differential misclassification. Differential misclassification may have biased results toward or away from the null, but in comparing multiple imputations with other methods for analyzing data with large percentages of missing values, multiple imputation produces less biased and more efficient estimates [18]. Additional limitations were the inability to calculate an odds ratio for maternal hormone use because no mothers of cases reported hormone use, and this study's failure to collect information on birth length, paternal age and pre-eclampsia/eclampsia which were associated with breast cancer in a meta-analysis [2]. In addition, this study was unable to assess effect modification by menopausal status owing to the small number of premenopausal cases (n = 39), which is of importance since Sanderson et al. [19] identified differing birth weight-breast cancer associations for premenopausal and postmenopausal women.

As far as this study is concerned, it is the first to investigate the association between perinatal factors and breast cancer among Hispanic women. Given the differing distributions of perinatal factors in Hispanic women relative to women of other ethnicities, it is important to include this group to further clarify the contribution of prenatal exposures to adult-onset diseases. This study was unable to categorize birth weight differently because 35% of women who were unable to report their exact birth weight reported it as less than 2500, 2500–3999 or 4000 g or more. However, a sensitivity analysis was performed comparing women who were first born with those who were second born (OR 1.03, 95% CI 0.61–1.75), third born (OR 0.99, 95% CI 0.56–1.74) and fourth born or higher (OR 0.91, 95% CI 0.59–1.38) which revealed a reduction in risk with higher birth order. Lastly, this study assessed confounding by a number of established breast cancer risk and protective factors, including mammography screening, which reduced the likelihood of detection bias.

Hispanic women have relatively low incidence rates of breast cancer although they possess some of the same risk factors as ethnic groups with higher incidence rates. As Hines et al. [14] suggest, the study of Hispanic women may help us disentangle the effect of the hormonal milieu on breast cancer. Confirmation of these findings in larger studies may assist in determining how hormonal mechanisms responsible for breast cancer differ by ethnicity.

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BRIEF REPORT

Type 2 diabetes and mammographic breast density among underserved women

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Abstract
Purpose We conducted a study of women recruited at Meharry Medical College, a historically black medical school, to investigate the relationship between diabetes and mammographic breast density.
Methods A total of 476 women completed in-person interviews, body measurements, and full-field digital mammograms on a Hologic mammography unit from December 2011 to February 2014. Average percent breast density for the left and right breasts combined was estimated using Quantra, an automated algorithm for volumetric assessment of breast tissue. The prevalence of type 2 diabetes was determined by self-report.
Results After adjustment for confounding variables, the mean percent breast density among premenopausal women with type 2 diabetes [$\hat{\mu}$ 13.8 %, 95 % confidence interval (CI) 11.6–15.9] was nonsignificantly lower than that of women without type 2 diabetes ($\hat{\mu}$ 15.9 %, 95 % CI 15.0–16.8) ($p = 0.07$); however, there was no association among postmenopausal women. The effect of type 2 diabetes in severely obese women (BMI $\geq$ 35) appeared to differ by menopausal status with a reduction in mean percent breast density in premenopausal women, but an increase in mean percent breast density in postmenopausal women which could have been due to chance.
Conclusions Confirmation of our findings in larger studies may assist in clarifying the role of the insulin signaling breast cancer pathway in women with high breast density.

Keywords Mammographic breast density · Type 2 diabetes · Cross-sectional study · Underserved

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Introduction

Type 2 diabetes has been identified as a weak risk factor for breast cancer, independent of obesity. Meta-analyses of the association between diabetes and breast cancer, consisting primarily of cohort studies, have reported summary relative risks (RRs) of approximately 1.20, with 95 % confidence intervals (CI) ranging from 1.12 to 1.30 [1–4]. Three of the four meta-analyses stratified by menopausal status at breast cancer diagnosis reported an increased risk of postmenopausal breast cancer associated with diabetes among women, but not among premenopausal women [2–4]. The increase in postmenopausal breast cancer risk associated with diabetes was also reported in a recent large cohort study conducted since these meta-analyses [5]. In another more recent large cohort study, Bowker et al. [6] reported that risk of breast cancer diagnosed among women at age 55 years or older, and presumably postmenopausal, was nonsignificantly increased for 0–3 months following diabetes diagnosis [hazard ratio (HR) 1.31, 95 % CI 0.92–1.86], but then returned to baseline from 3 months to 10 years following diabetes diagnosis (HR 1.00, 95 % CI 0.90–1.11). The authors concluded that the initially elevated postmenopausal breast cancer risk may have been due to detection bias.

High mammographic breast density is a well-established risk factor for breast cancer. Depending on how high mammographic breast density is defined, the range of RRs for breast cancer is around 4–6 [7]. In a meta-analysis of 42 studies, the group of women whose fibroglandular tissue comprised ≥75 % of breast tissue had a summary RR for breast cancer of 4.64 (95 % CI 3.64–5.91) relative to women with <5 % [8]. Several breast cancer risk factors that affect the growth (proliferation and apoptosis) and/or differentiation of breast tissue, such as parity, menopause, hormone replacement therapy, body mass index, and hormone levels, are also associated with mammographic breast density [7, 9, 10]. Few studies have assessed breast density among Black or Hispanic women. In comparison with White women, Black women have been reported to have denser breasts [11–13], breasts of similar density [14, 15], or less dense breasts [16], while Hispanic women have been reported to have breasts of similar density [11, 16]. These studies varied in regard to the age of the study subjects and the methods used to assess breast density.

Although mammographic breast density is thought to be an intermediate phenotype of breast cancer [17], very few studies have investigated the association between diabetes and mammographic breast density. Diabetes may play a role in mammographic breast density through the insulin signaling pathway given that insulin has been linked with low breast density in premenopausal women [18, 19]. We conducted a study of women recruited at Meharry Medical College, a historically black medical school, to investigate the relationship between diabetes and mammographic breast density.

Materials and methods

Study design

We conducted a clinic-based cross-sectional study of underserved women aged 40–79 years recruited at Meharry Medical College, a historically black medical school, between December 2011 and February 2014 to investigate mammographic breast density and its relation with potential breast cancer pathways including insulin [18, 19], insulin-like growth factor [10, 20], and adipocytokine [19, 21]. The present study focuses on the insulin signaling pathway by investigating the association between type 2 diabetes and mammographic breast density. Subjects were eligible if they were underserved meaning their primary place of health care was a safety net clinic. Women were recruited by placing flyers around the campus, and at health fairs and local community agencies. The flyer described the study and asked women to provide contact information if they were interested in participating. Project staff telephoned each woman to evaluate eligibility and to schedule a study appointment. The Institutional Review Boards of Meharry Medical College and Vanderbilt University approved this study's protocol. After informed consent was obtained, women provided a fasting blood sample, underwent body measurements (height, weight, waist, hips, percent body fat) and a digital screening mammogram, and completed an in-person interview on demographics, lifestyle factors, personal health history, family history of cancer and other chronic diseases, adult weight history, diet, and health literacy. Body mass index (BMI) (kg/m^2) and waist-to-hip ratio (WHR) were calculated from body measurements, and percent body fat was estimated from a Body Fat Monitor Scale.

Study population

Women who were pregnant, unable to comprehend study materials, or had a history of cancer, breast augmentation or reduction, symptoms of a breast disorder, or a focal dominant lump were ineligible. Premenopausal women were asked the date of their last menstrual period so their appointment could be scheduled during the follicular phase (1–14 days) of their menstrual cycle when their breast tissue is less dense. The day prior to their appointment, women were telephoned and reminded to observe a 10-h fast for their blood draw the following morning. Of the 479 women recruited, exclusions due to incomplete interviews ($n = 4$), type 1 diabetes ($n = 11$), and unknown age at diabetes diagnosis ($n = 1$) resulted in 175 premenopausal women and 288 postmenopausal women for analysis.

Assessment of breast density

A trained radiologic technician completed full-field digital screening mammograms on a Hologic mammography unit that uses selenium direct capture technology to eliminate light diffusion completely for perfect clarity and image quality. Our study radiologist (ACD) estimated average percent breast density, defined as the ratio of estimated fibroglandular tissue volume to total breast volume, for the left and right breast combined using Quantra software and assigned Breast Imaging Reporting and Data System (BI-RADS) categories of 0–4 represented by increasing density [22]. Subjects with abnormal mammograms (BI-RADS = 0: additional imaging evaluation, $n = 45$; BI-RADS = 3: probably benign finding, $n = 3$; BI-RADS = 4: suspicious abnormality, $n = 2$) were notified immediately by certified mail, while subjects with normal mammograms were notified of their results within 30 days.

Assessment of type 2 diabetes status

To define diabetes, we used self-reported diabetes from the questionnaire. Women were considered diabetic if they

responded "Yes" to the question "Did a doctor or other health care provider ever tell you that you had diabetes, or high sugar in your blood or urine?" on the questionnaire. Women who indicated they had diabetes "Only during pregnancy" on the questionnaire were categorized as non-diabetic. On the questionnaire, women who reported that they had diabetes were then asked how old they were when they were first told that they had diabetes and whether they used pills or insulin injections to control their diabetes. Women who indicated their age at diabetes diagnosis was ≤30 years were considered to have type 1 diabetes and were excluded from analysis [23]. For the medication analysis, women who used pills and then switched to insulin to control diabetes were classified as having used insulin.

We conducted a validation study of self-reported diabetes using c-peptide (a biomarker of insulin secretion) which was measured in fasting serum samples using chemiluminescence technology-based assay kits on a proprietary automated moderate complexity endocrine panel (Immulite 1000) according to the manufacturers' instructions (Siemens, Dallas, TX). The calculated sensitivity of the assay ($n = 6$) was 0.03 ng/tube, and the intra-assay coefficients of variation (CVs) for levels 1, 2, and 3 controls ($n = 10$/level of control) were 2.2, 3.4, and 3.0 %, respectively, within the range of acceptable sensitivity and CVs [24]. The inter-assay CVs were not available because the sera were batch analyzed in two assays. For the validation study, women were considered to have diabetes if their fasting serum c-peptide was >2.0 ng/mL [25].

Statistical analysis

Statistical analyses were performed in SAS version 9.2. Linear regression was used to estimate mean percent breast density by type 2 diabetes status, while adjusting for confounding variables [26]. We stratified by menopausal status a priori, since fibroglandular breast tissue decreases during the menopausal transition [27]. Interaction terms, the product of diabetes and race/ethnicity (White, Black, Hispanic) and BMI (<35, ≥35), were added to linear regression models, and likelihood ratio tests were performed to test for effect measure modification. Covariates examined as potential confounders of the relationship between diabetes and mean percent breast density included race/ethnicity, age, education, family history of breast cancer, family history of diabetes, age at menarche, parity, age at first pregnancy, oral contraceptive use, smoking, alcohol intake, physical activity, BMI, WHR, percent body fat, age at menopause, and hormone replacement therapy (HRT) use. Confounders were examined as categorized in Table 1 with the exception of age, BMI, WHR, and percent body fat which were examined continuously.

Table 1 Demographic characteristics and breast cancer risk factors for participants by menopausal status

Characteristic	Premenopausal ($n = 175$)		Postmenopausal ($n = 288$)	
	n	Percent	*n*	Percent
Race				
White	36	20.6	74	25.7
Black	79	45.1	172	59.7
Hispanic	60	34.3	42	14.6
Age (years)				
40–49	157	89.7	59	20.5
50–64	18	10.3	178	61.8
65–79	0	0.0	51	17.7
Education				
<High school	49	28.3	65	22.6
High school graduate	41	23.7	74	25.7
Some college	55	31.8	91	31.6
College graduate	28	16.2	58	20.1
Missing	2		0	
Family history of breast cancer				
No	116	66.3	184	64.8
Yes	50	28.5	91	32.0
Adopted	8	4.6	8	2.8
Don't know	1	0.6	1	0.4
Missing	0		4	
Family history of diabetes				
No	58	33.1	77	26.9
Yes	109	62.3	200	69.9
Adopted	8	4.6	8	2.8
Don't know	0	0.0	1	0.4
Missing	0		2	
Age at menarche (years)				
≤12	85	48.6	143	49.7
13	35	20.0	68	23.6
>13	55	31.4	77	26.7
Number of full-term pregnancies				
0	23	13.2	23	8.0
1–2	43	24.7	90	31.4
3–4	72	41.4	109	38.0
≥5	36	20.7	65	22.6
Missing	1		1	
Age at first pregnancy (years)[a]				
<30	134	89.3	247	95.0
≥30	16	10.7	13	5.0
Missing	1		4	
Oral contraceptive use				
No	50	28.9	77	26.7
Yes	123	71.1	211	73.3
Missing	2		0	

Table 1 continued

Characteristic	Premenopausal ($n = 175$)		Postmenopausal ($n = 288$)	
	n	Percent	*n*	Percent
Smoking				
No	104	59.4	116	40.4
Yes	71	40.6	171	59.6
Missing	0		1	
Alcohol intake				
No	99	56.9	135	47.2
Yes	75	43.1	151	52.8
Missing	1		2	
Physical activity				
None	54	30.9	97	33.8
Moderate	63	36.0	116	40.4
Strenuous	58	33.1	74	25.8
Missing	0		1	
Body mass index				
<25	25	14.4	52	18.1
25–29.9	56	32.4	77	26.8
30–34.9	41	23.7	75	26.2
≥35	51	29.5	83	28.9
Missing	2		1	
Waist-to-hip ratio				
<0.84	49	28.3	66	23.0
0.84–0.88	40	23.1	75	26.1
0.89–0.92	51	29.5	64	22.3
≥0.93	33	19.1	82	28.6
Missing	2		1	
% Body fat				
<37.9	45	26.5	64	22.6
37.9–43.0	51	30.0	67	23.7
43.1–47.2	34	20.0	78	27.6
≥47.3	40	23.5	74	26.1
Missing	5		5	
Age at menopause (years)[b]				
<50			218	75.7
50–54			54	18.7
≥55			12	4.2
Don't know			4	1.4
Hormone replacement therapy use[b]				
No			199	69.3
Yes			88	30.7
Missing			1	

[a] Among parous

[b] Among postmenopausal

Variables were considered confounders if their addition to the model changed the unadjusted mean percent breast density by 10 % or more. There was no evidence of statistical interaction for race/ethnicity or BMI; however, we present results for type 2 diabetes stratified by BMI since the effect of diabetes on mean percent breast density appears to be most pronounced among severely obese women. In addition, we stratified by menopausal status and adjusted for race/ethnicity, age, and BMI (as needed), and additionally, for HRT use among postmenopausal women which met our criteria for model inclusion. Adjustment for WHR and percent body fat did not meet our criteria for confounding. For our validation study of self-reported diabetes, we used serum c-peptide as the gold standard and calculated sensitivities and specificities and their respective confidence intervals as measures of validity. Lastly, we performed a sensitivity analysis by examining our findings with and without the inclusion of 50 women with abnormal mammograms and our results were similar.

Results

Table 1 presents the demographic characteristics and breast cancer risk factors for participants by menopausal status. In both premenopausal and postmenopausal groups, we observed a high prevalence of several breast cancer risk factors including family history of breast cancer, younger age at menarche, alcohol intake, no physical activity, and high body measurements. The percentage of all women reporting a family history of diabetes was extremely high (premenopausal 62.3 %; postmenopausal 69.9 %).

Table 2 presents mean percent breast density associated with type 2 diabetes by menopausal status. After adjustment for confounding variables, the mean percent breast density among premenopausal women with type 2 diabetes ($\hat{\mu}$ 13.8 %, 95 % CI 11.6–15.9) was nonsignificantly lower than that of women without type 2 diabetes ($\hat{\mu}$ 15.9 %, 95 % CI 15.0–16.8) ($p = 0.07$); however, there was no association among postmenopausal women. Among severely obese (BMI ≥ 35) premenopausal women, the mean percent breast density was nonsignificantly lower in women with diabetes ($\hat{\mu}$ 10.8 %, 95 % CI 8.3–13.2) than in women without diabetes ($\hat{\mu}$ 13.1 %, 95 % CI 11.9–14.4) ($p = 0.07$). However, the opposite was true in severely obese postmenopausal women with a significantly higher mean percent breast density in women with diabetes ($\hat{\mu}$ 12.8 %, 95 % CI 11.8–13.8) than in women without diabetes ($\hat{\mu}$ 11.1 %, 95 % CI 10.1–12.0) ($p = 0.01$). While premenopausal women whose diabetes was diagnosed at least 10 years ago had lower mean percent breast density than women diagnosed <5 years ago, the opposite was true for postmenopausal women. There was no effect of the use of insulin or pills among diabetics on mean percent breast density.

To ascertain misclassification of self-reported diabetes, we conducted a validation study using fasting serum

Table 2 Mean percent breast density associated with type 2 diabetes by menopausal status

Characteristic	Premenopausal				Postmenopausal			
	n	Mean % density[a]	95 % CI	*p* value	*n*	Mean % density[b]	95 % CI	*p* value
Type 2 diabetes								
Overall								
No	151	15.9	15.0–16.8		221	13.0	12.3–13.6	
Yes	24	13.8	11.6–15.9	0.07	67	13.1	12.1–14.2	0.78
BMI < 35								
No	108	17.0	15.8–18.2		167	13.5	12.7–14.3	
Yes	14	15.1	11.9–18.2	0.25	37	13.0	11.5–14.5	0.49
BMI ≥ 35								
No	41	13.1	11.9–14.4		54	11.1	10.1–12.0	
Yes	10	10.8	8.3–13.2	0.07	29	12.8	11.8–13.8	0.01
Times since diabetes diagnosis (years)[c]								
<5	16	14.2	12.4–16.0	Referent	31	12.3	10.9–13.8	Referent
5–9	4	10.5	6.7–14.3	0.07	17	11.7	9.8–13.6	0.55
≥10	4	10.2	6.4–14.0	0.05	19	14.8	13.0–16.5	0.03
Diabetes medications[c]								
None	6	13.9	11.0–16.9	Referent	12	13.4	11.2–15.6	Referent
Insulin	11	13.7	11.2–16.2	0.90	30	12.3	10.8–13.9	0.42
Pills	7	10.9	7.6–14.2	0.16	25	13.3	11.5–15.1	0.94

[a] Adjusted for race/ethnicity, age, and BMI (as needed)

[b] Adjusted for race/ethnicity, age, BMI (as needed) and HRT use

[c] Among self-reported diabetics

c-peptide available for 95 % of subjects as the gold standard. Results indicated very low sensitivity (22.8, 95 % CI 18.0–28.3) and high specificity (83.2, 95 % CI 77.2–87.9) of self-report of diabetes in comparison with serum c-peptide. The total percentage of women whose c-peptide level indicated diabetes (58.0 %) was 37.8 % higher than the percentage of women who self-reported diabetes (20.2 %). This percentage is higher than the estimated 27.8 % of undiagnosed diabetes in the USA [28], but may be due to the high rates of obesity (BMI 30–34.9; 25 %) and severe obesity (BMI ≥ 35; 29 %) in our study population.

Discussion

We found a nonsignificantly lower mean percent density associated with self-reported diabetes among premenopausal women, but no association in postmenopausal women after continuous adjustment for BMI. This finding is in agreement with two studies of self-reported diabetes and mammographic breast density. Robidoux et al. [18], in a study of Southwestern Native American women, classified breast density using BI-RADS categories analog mammograms and found that self-reported diabetes was associated with lower breast density (moving up from one BI-RADS category to the next) in premenopausal ($p = 0.0032$) but not in postmenopausal women ($p = 0.3178$). Sellers et al. [29], in a study of primarily postmenopausal White women in Minnesota, found no association between self-reported type 2 diabetes and breast density based on a computer-assisted thresholding program (Cumulus) [30] of analog mammograms in premenopausal or postmenopausal women. However, these investigators did identify a positive association between diabetes and breast cancer.

This finding is in partial agreement with two other studies that investigated c-peptide levels and breast density which found overall or within strata of menopausal status [31, 32]. Diorio et al. [31], in a study of primarily White women in Quebec City, found no association between non-fasting c-peptide levels and breast density based on the Cumulus thresholding program after adjustment for BMI and WHR ($p = 0.41$), and after stratification by menopausal status ($p = 0.94$). Ahern et al. [32], in a case–control study nested within the Nurses' Health Study cohorts, found no association between fasting c-peptide levels and breast density measured using Cumulus after adjustment for BMI and WHR in premenopausal and postmenopausal women combined and within strata of menopausal status. As was the case with Sellers et al. [29], these investigators did identify a positive association between diabetes and breast cancer.

Among self-reported diabetics in our study, premenopausal women whose diabetes was longer standing had lower mean percent breast density than women diagnosed more recently. To our knowledge, no other study has investigated breast density as it relates to the time since diabetes diagnosis. Our failure to find an effect of diabetes

treatment on breast density may have been due to limited statistical power, but was unexpected given the recent interest in utilizing metformin, one of the most common oral diabetes medications, as a breast cancer chemopreventive agent [33], particularly in postmenopausal women [34]. To date, one study has investigated the effect of metformin and breast density in postmenopausal women and reported a decrease in 7 of 14 women after 10.5 months of use that was more pronounced in women with no signs of metabolic syndrome [35].

Our study was potentially limited by selection bias since our sample was one of convenience. In addition, statistical power was limited, especially when we stratified by both menopausal status and BMI, so these results should be interpreted with caution. Also misclassification of breast density could have affected our results since we used Quantra, a fairly new automated algorithm for volumetric assessment of breast tissue, rather than the standard computer-assisted Cumulus thresholding program. To date, the validity of Quantra has yet to be established. In comparing Quantra with magnetic resonance imagining (MRI), Wang et al. [36] reported lower median percent breast density with Quantra [22.0 %, interquartile range (IQR) 14.0 %] than with MRI (24.0 %, IQR 36.0 %). Ciatto et al. [37] reported systematically lower percent breast density with Quantra compared with visual classification using BI-RADS by eleven experienced radiologists, but the authors maintained that its reproducibility makes it preferable to visual classification. Engelken et al. [38] reported a Pearson correlation coefficient of 0.920 ($p < 0.05$) for serial digital mammograms using Quantra software on the same unit within a 24-month period.

Very few epidemiologic studies of breast density have utilized full-field digital mammograms with Quantra software for comparison with our study. The mean breast density (19.7 %, range 8.5–48.5 %) and age (59 years, range 49–81 years) of an English study of premenopausal and postmenopausal women combined [39] were higher than that of our study (breast density 14.1 %, range 6.5–34.0 %; age 51 years, range 40–76 years). In a German study, Hammann-Kloss et al. [40] reported median breast densities for women of <46 years (28 %, IQR 15.0 %), 46–55 years (23.0 %, IQR 15.3 %), and >55 years (16.0 %, IQR 10.0 %) that were higher than those of our study (<46 years 15.0 %, IQR 8.25 %; 46–55 years 12.5 %, IQR 5.5 %; >55 years 11.5 %, IQR 3.75 %). Both of these findings may have been due to chance or due to the high prevalence of obesity, and therefore less dense breasts, in our population.

Strengths of our study included the high rates of diabetes in our population, a priori stratification by menopausal status, adjustment for known confounders, the validation study of self-reported diabetes, and the examination of findings with and without women who had abnormal mammograms. To our knowledge, our study is the first to identify a suggested association between type 2 diabetes and mammographic breast density in severely obese women that appeared to operate in opposite directions in premenopausal and postmenopausal women which could have been due to chance. Most studies of diabetes and breast density have only identified weak associations in premenopausal women suggesting that the link between diabetes and breast cancer is not mediated through breast density. Confirmation of our findings in larger studies may assist in clarifying the role of the insulin signaling breast cancer pathway in women with high breast density and ultimately target those women who will benefit most from primary and secondary prevention.

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Conflict of interest The authors declare that they have no conflict of interest.

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Results of the Breast Density Study

We have completed data collection for the Breast Density Study (the mammogram study) in which you participated during the last few years. Dr. Maureen Sanderson along with the study staff wish to thank you again for your participation. We also would like to share with you some results. Breast cancer is the most common cancer among American women and, except for skin cancers, is the second leading cause of death in women after lung cancer. To reduce death from breast cancer, a better understanding of risk factors is important. Recently, studies have shown that breast density, that is the amount of fibrous and glandular tissue (not fat) in a woman's breast, has been found to increase her risk of breast cancer. In this study, as you will remember, we also took blood and body measurements and asked you some questions about your health and diet. We are looking to see if any of these things might also be associated with dense breasts. Without your gracious participation, studies like these would not be possible.

MEHARRY MEDICAL COLLEGE

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Wishing You a Happy Thanksgiving and Joyous Holiday Season!

Before discussing the results, there are two important points to make: First, no one study can tell us for sure what causes or might increase the risk of cancer. All research needs to be repeated more than once to confirm the results. Second, this study type is one that looks at groups of people and does not tell us what will happen to any single person. For example, we know that smoking can increase a person's risk of lung cancer, but not everyone who smokes will get lung cancer and indeed some persons who have never smoked will get lung cancer.

During the more than 2 years of this study, 464 women completed in-person interviews, body measurements and digital mammograms. We separated the women by menopausal status as we know that a woman's breast density will decrease after menopause. The table below describes some of the characteristics of our participants.

Characteristic	Premenopausal Number=175		Postmenopausal Number=289	
	Number	%	Number	%
Race/Ethnicity				
African-American	79	45.1	172	59.5
White	36	20.6	75	26.0
Hispanic	60	34.3	42	14.5
Age				
40-49 years	157	89.7	59	20.4
50-64 years	18	10.3	179	61.9
65-79 years	0	0.0	51	17.7
Breast Cancer in Family				
No	116	66.3	184	64.6
Yes	50	28.5	92	32.3
Don't Know/Missing	9	5.2	13	3.1
Body mass index (weight/height)				
<25 (normal weight)	25	14.4	52	18.1
25-29.9 (overweight)	56	32.4	77	26.7
30-34.9 (obese)	41	23.7	75	26.0
35+ (extremely obese)	51	29.5	84	29.2
Missing	2		1	

In other research, diabetes has been identified as a weak risk factor for breast cancer while high breast density has been identified as a strong risk factor. Therefore, we looked at the association of Type 2 diabetes and breast density by menopausal status. In our study, the average percent breast density of premenopausal and postmenopausal women was similar regardless of whether or not they had diabetes.

Characteristic	Premenopausal Number=175		Postmenopausal Number=289	
	Number	Average % density	Number	Average % density
Type 2 diabetes				
No	99	14.8	104	13.4
Yes	76	14.6	184	12.9

We have many more questions we will be attempting to answer about breast density, diet and exercise. Your participation has been very important to these efforts. If you would like more information or have any questions, please feel free to contact Dr. Maureen Sanderson, Meharry Medical College, Department of Family and Community Medicine, 1005 Dr. D.B. Todd Jr. Blvd., Nashville, TN 37208, Phone: 615-321-2977, E-mail: msanderson@mmc.edu.

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Original Research

Increased vitamin D and calcium intake associated with reduced mammographic breast density among premenopausal women

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ABSTRACT

Vitamin D has been identified as a weak protective factor for postmenopausal breast cancer (relative risk, ~0.9), whereas high breast density has been identified as a strong risk factor (relative risk, ~4-6). To test the hypothesis that there is an association between vitamin D intake, but not circulating vitamin D levels, and mammographic breast density among women in our study, we conducted a cross-sectional study of 165 screening mammography patients at Nashville General Hospital's Breast Health Center, a public facility serving medically indigent and underserved women. Dietary and total (dietary plus supplements) vitamin D and calcium intakes were estimated by the Harvard African American Food Frequency Questionnaire, and blood samples were analyzed for 25-hydroxyvitamin D. Average percent breast density for the left and right breasts combined was estimated from digitized films using an interactive thresholding method available through Cumulus software. After statistical adjustment for age, race, and body mass index, the results revealed that there were significant trends of decreasing breast density with increasing vitamin D and calcium intake among premenopausal but not among postmenopausal women. There was no association between serum vitamin D and breast density in premenopausal or postmenopausal women. Confirmation of our findings in larger studies may assist in clarifying the role of vitamin D in breast density.

Abbreviations: BMI, body mass index; MI, melanin index; RR, relative risk; MI, melanin index.

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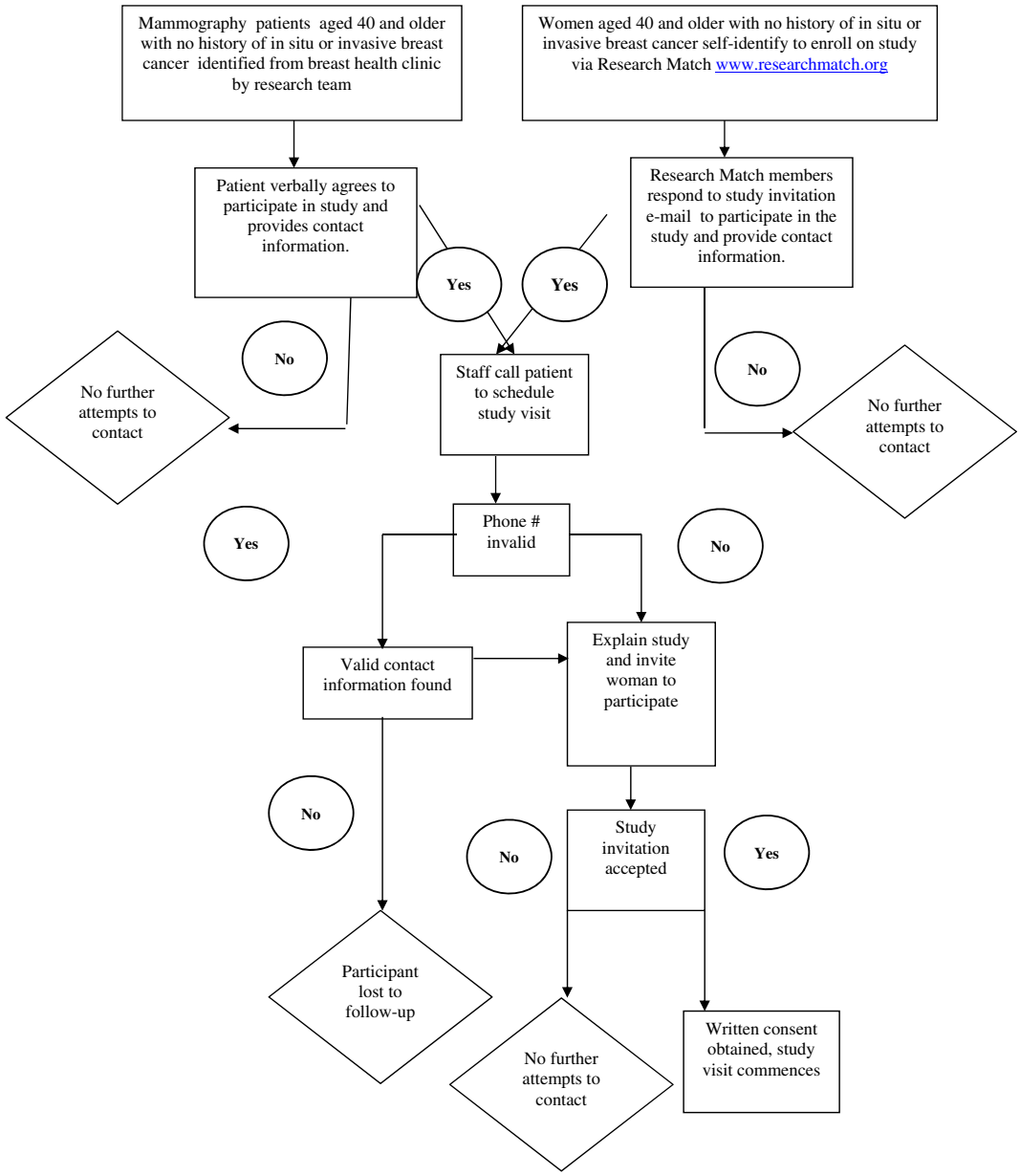

Figure – Methods to recruit and deliver research study to participants for vitamin D and mammographic breast density study.

1. Introduction

Vitamin D has been identified as a weak protective factor for postmenopausal breast cancer (relative risk [RR], ~0.9) [1], whereas high mammographic breast density, defined here as the percentage of fibroglandular tissue in the breast, has been identified as a strong risk factor (RR, ~4-6) [2]. Vitamin D has antiproliferative and prodifferentiation effects in normal breast tissue and could have direct or indirect influences on breast tissue composition [3]. A recent systematic review of the association between vitamin D and mammographic breast density included comparisons of 4 studies of vitamin D intake and 4 studies of circulating vitamin D levels [4]. Two of the studies of vitamin D intake reported significantly higher adjusted mean percent breast density for the highest vs lowest intake among all and premenopausal Hispanic women [5] and among all premenopausal women [6], but in 1 study, the reverse was true for postmenopausal women [7], and the final study showed no association [8]. None of the 4 studies of circulating vitamin D levels conducted among premenopausal and postmenopausal women reported significant findings for adjusted mean percent density [9-12]. The aforementioned studies did not investigate the vitamin D and mammographic breast density association in black women separately; however, findings in studies among mixed race populations are varied [7,9,12-14].

In view of the emerging evidence that vitamin D might be a breast cancer risk reduction factor and the noted predilection for vitamin D deficiency in African American women, we propose to examine the association of vitamin D with breast density, an established marker of increased breast cancer risk.

Table 1 – Demographic characteristics and breast cancer risk factors of participants by menopausal status [a]

Characteristic	Premenopausal (n = 57)		Postmenopausal (n = 106) [b]	
	n	%	n	%
Mean age (y)	57	46.2 ± 3.8	106	55.6 ± 7.0
Race				
White	30	52.6	50	47.2
Black	27	47.4	56	52.8
Educational level				
≤High school	21	36.8	48	45.7
≥Some college	36	63.2	57	54.3
Missing	0		1	
Marital status				
Married	18	31.6	30	29.9
Single/living with a partner	20	35.1	52	44.5
Divorced/widowed/separated	19	33.3	23	25.6
Missing	0		1	
Household income				
≤$19 999	26	47.3	55	53.4
$20 000-$39 999	12	21.8	26	25.2
$40 000-$59 999	8	14.5	10	9.7
≥$60 000	9	16.4	12	11.7
Missing	2		3	
Age at menarche (y)				
≤12	31	54.4	56	52.8
13	9	15.8	24	22.7
>13	17	29.8	26	24.5
No. of full-term pregnancies				
0	16	28.1	23	21.7
1	7	12.3	21	19.8
2	16	28.1	28	26.4
≥3	18	31.5	34	32.1
Age at first pregnancy (y) [c]				
<30	39	95.1	79	95.2
≥30	2	4.9	4	4.8
Oral contraceptive use				
No	12	21.1	26	24.5
Yes	45	78.9	80	75.5
Diabetes				
No	50	87.7	95	89.6
Yes	7	12.3	11	10.4
Type 1	1	1.8	3	2.8
Type 2	5	8.7	8	7.6
Don't know	1	1.8	0	0.0
Smoking				
No	22	39.3	41	38.7
Yes	34	60.7	65	61.3
Missing	1		0	

Characteristic	Premenopausal (n = 57)		Postmenopausal (n = 106) [a]	
	n	%	n	%
Alcohol intake (g) [d]				
None	24	42.1	46	43.4
<4.63	21	36.8	30	28.3
≥4.63	12	21.1	30	28.3
BMI (kg/m^2)				
<25	11	19.3	22	20.8
25-29.9	13	22.8	32	30.2
30-34.9	13	22.8	21	19.8
≥35	20	35.1	31	29.2
Waist-hip ratio				
<0.77	17	29.8	33	31.1
0.77-0.81	20	35.1	22	20.8
0.82-0.85	5	8.8	29	27.4
≥0.86	15	26.3	22	20.8

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Table 1 (continued)

Characteristic	Premenopausal (n = 57) n	Premenopausal (n = 57) %	Postmenopausal (n = 106) [b] n	Postmenopausal (n = 106) [b] %
MI				
<0.99	20	39.4	33	32.0
0.99-1.78	16	29.1	36	35.0
≥1.79	19	34.5	34	33.0
Missing	2		3	
Season of blood draw				
Summer	26	45.6	39	36.8
Spring	17	29.8	33	31.1
Fall	10	17.6	25	23.6
Winter	4	7.0	9	8.5
Age at menopause (y) [e]				
<50			78	73.6
50-54			24	22.6
≥55			4	3.8
Hormone replacement therapy use [e]				
No			63	59.4
Yes			43	40.6

[a] For premenopausal and postmenopausal groups, means ± SD are presented for continuous variables, and percentages are presented for categorical variables.
[b] Information on menopausal status was missing for 2 women.
[c] Among parous women.
[d] From food frequency questionnaire.
[e] Among postmenopausal women.

The RR associated with highly dense breasts is greater than most traditional risk factors such as nulliparity and early menarche, making it an attractive target for intervention. Unlike most other breast cancer risk factors, breast density may be influenced by alterations in lifestyle [15], possibly including improvement of vitamin D status.

We conducted a cross-sectional analysis of diet and serum vitamin D in relation to breast density in a screening population with a large proportion of medically underserved African American women. Medically underserved populations include groups of persons who face economic, cultural, or linguistic barriers to health care. Our primary research question was to examine whether low serum vitamin D is associated with breast cancer through its association with greater breast density. The study hypothesis was that there would be an association between vitamin D intake, but not circulating vitamin D levels, and mammographic breast density among women in our study. The main objective of this project was to examine whether vitamin D, as measured in the serum, effectively discriminates women with high- and low-risk breasts based on mammographic findings. In order to examine this hypothesis, we collected information on diet and lifestyle and obtained blood samples to store the serum and DNA. Plasma vitamin D metabolite concentrations were examined among all of the women and were related to levels of breast density measured in digitized mammogram. Overall, our goals are to contribute important new information on a potentially modifiable breast cancer risk factor, to address disparities in breast cancer research in an underserved African American population that is at high risk for both vitamin D deficiency and breast cancer, and to provide critical pilot data for more definitive full-scale studies.

2. Methods and materials

We conducted a cross-sectional study to investigate the relationship between vitamin D and mammographic breast density. Eligible participants were medically underserved women aged 40 years and older with no history of in situ or invasive breast cancer. The women were recruited from a county hospital mammography center located in a federally designated medically underserved area [16]. An additional source of recruitment was ResearchMatch.org, a portal for linking interested individuals with active research studies [17]. A total of 165 women completed in-person interviews, blood draws, body measurements, and analog mammograms from December 2009 to February 2011 (see Figure).

2.1. Study procedures

In-person interviews were administered to assess lifestyle, sun exposure, family history of cancer, diet, reproductive history, demographic characteristics, and established breast cancer risk factors. The Harvard African American Food Frequency Questionnaire and study-specific questions modified from the Collaborative Breast Cancer Study Questionnaire [18] were used to categorize dietary intake and supplement use of vitamin D and calcium into tertiles.

The Harvard African American Food Frequency Questionnaire was used because it is a validated instrument that quantitatively measures dietary vitamin D. Serum vitamin D levels were assayed from blood samples [19,20] and categorized into tertiles and as deficient (<20 ng/mL). Plasma vitamin D metabolite concentrations were determined by

Table 2 – Levels of breast density associated with dietary vitamin D and calcium by menopausal status [a]

Characteristic	Premenopausal (n = 57)			Postmenopausal (n = 106)		
	n	% density [b]	95% CI	n	% density [b]	95% CI
Total vitamin D intake, IU/d						
<191.56	12	33.0	23.9-42.1	36	20.8	15.8-25.8
191.55-568.8	24	30.9	24.4-37.4	36	20.0	14.8-25.1
≥568.9	21	23.9	17.1-30.7	34	16.5	10.8-22.2
P for trend			.03			.67
Vitamin D from food sources, IU/d						
<124.57	16	32.7	24.5-40.8	32	20.2	15.0-25.5
124.57-243.86	21	31.0	24.2-37.8	36	16.9	11.6-22.1
≥243.86	20	23.4	16.3-30.4	38	20.6	15.5-25.7
P for trend			.02			.33
Total calcium intake, IU/d						
<730.1	13	30.2	21.1-32.2	33	20.4	15.2-25.7
730.1-1405.69	22	31.0	24.0-38.0	37	21.1	16.0-26.1
≥1405.69	22	25.7	19.0-32.5	36	16.1	10.8-21.4
P for trend			.003			.51

Abbreviation: CI, confidence interval.
[a] Values are means expressed as percentages and 95% confidence intervals.
[b] Analysis performed using linear regression adjusted for age, race, and BMI.

radioimmunoassay, without knowledge of the mammographic findings of the subject. 25-Hydroxyvitamin D was used as the marker of vitamin D status [21]. Vitamin D level results were classified into deficient, insufficient, and sufficient vitamin D levels [20,22]. Anthropometric measurements included height and weight used to calculate body mass index (BMI); circumference of the waist and hips used to calculate waist-hip ratio; and, for a subsample of women triceps, suprailiac and thigh skinfold thickness used to calculate body fat distribution [23].

Melanin index (MI) was estimated by measuring skin color at the underarm with the use of a Konica Minolta Portable Spectrophotometer (model CM-2600d). This device produces a direct and reproducible MI (Konica Minolta, Ramsey, NJ, USA). The MI is the inverse amount of back-reflected light over the visible spectrum of wavelengths estimating light absorbed, accounting for concentration of cutaneous melanin. The MI provides a proxy estimate of vitamin D absorption through sun exposure [24]. Average percent breast density for the left and right breasts combined was assessed from digitized films of analog mammograms using an interactive thresholding method available through Cumulus software which is a well-validated computer-assisted planemetry program [25,26]. Reliability of density measurements with this method is reported to be 90% or greater [15,25]. Mammographic breast density measures were available for 154 women. The institutional review boards of the participating institutions approved this study's protocol.

2.2. *Statistical analyses*

Statistical analyses were performed in SAS version 9.2 (Cary, NC, United States). We calculated the power of the study to detect a range of odds ratios assuming 550 women are enrolled, of whom 40% will have greater than or equal to 50% breast density (the "cases"). We further assume conservatively that 20% of those with lower breast density (the "controls") will have low serum vitamin (≤16 ng/mL). Under these assumptions, power will be adequate (≥80%) to detect moderate main effect associations (odds ratios ≤0.57/≥1.75) at a

Table 3 – Percent breast density associated with serum vitamin D levels and vitamin D deficiency by menopausal status [a]

Characteristic	Premenopausal (n = 57)			Postmenopausal (n = 106)		
	n	% density [b]	95% CI	n	% density [b]	95% CI
Serum vitamin D levels, ng/mL						
<17.55	20	29.7	21.3-38.0	35	19.4	13.5-25.4
17.56-28.6	16	26.4	17.7-35.2	38	23.4	17.5-29.3
≥28.7	21	25.0	16.3-33.6	33	20.2	14.1-26.3
P for trend			.69			.20
Vitamin D deficiency (<20 ng/mL)						
No	32	24.6	17.1-32.0	61	22.4	17.6-27.2
Yes	25	29.9	22.5-37.2	45	18.9	13.5-24.2
P			.31			.31

[a] Values are means expressed as percentages and 95% confidence intervals.
[b] Analysis performed using linear regression adjusted for age, race, BMI, and season of blood draw.

P value of less than .05. We did not succeed in recruiting the planned number of subjects (n = 550); however, the precision of our estimates from the study actually performed can be inferred from our 95% confidence intervals.

We used linear regression to estimate the mean percent breast density associated with vitamin D intake and circulating vitamin D levels while adjusting for confounding variables [27]. Race, age, educational level, marital status, household income, age at menarche, number of full-term pregnancies, age at first pregnancy, oral contraceptive use, diabetes, smoking, alcohol intake, BMI, waist-hip ratio, MI, season of blood draw, age at menopause, and hormone replacement therapy were evaluated as potential confounders. Variables were considered confounders if their addition to the model changed the unadjusted mean by 10% or more. Findings are presented separately by menopausal status because breast density decreases during the menopausal transition [28]. We adjusted for age, race, and BMI, which were confounders in our data, and additionally adjusted serum vitamin D analyses for season of blood draw.

3. Results

Premenopausal women were more likely to be white; highly educated; and divorced, widowed, or separated and to have a higher household income than postmenopausal women. Premenopausal women were also more likely to have undergone menarche at a later age, to be nulliparous and obese, and to have a lower MI. Table 1 further illustrates the demographic characteristics and breast cancer risk factors of participants by menopausal status.

We observed a significant trend of decreasing breast density with increasing total vitamin D intake, vitamin D from food sources, and total calcium intake after adjustment for age, race, and BMI in premenopausal women only. Similar associations were not observed among postmenopausal women. Table 2 presents mean breast density associated with dietary vitamin D and calcium intake by menopausal status.

Serum vitamin D was unrelated to mammographic breast density overall, although a nonsignificant pattern of higher density with lower serum levels of vitamin D or a deficient state (<20 ng/mL) was observed in premenopausal women (n = 57), after adjustment for age, race, BMI, and season of blood draw. Table 3 further illustrates mean percent breast density in association with serum vitamin D levels and vitamin D deficiency by menopausal status.

4. Discussion

In the present study, our research hypothesis was accepted: increasing vitamin D intake was associated with reduced mammographic breast density in premenopausal women. Higher intake of calcium was also associated with more favorable patterns of breast density. Higher serum levels of vitamin D were also inversely associated with breast density in premenopausal women, although findings were based on limited numbers of women and did not attain statistical significance. No similar associations were present among postmenopausal women. Our findings are consistent with those of Diorio et al and others [4,5,29-31], which showed a benefit of these nutrients in association with breast density in premenopausal women only. Similarly, Bertone-Johnson et al [7,32] found evidence of protective association with supplemental vitamin D in analyses restricted to younger women with high mammography breast density or those at higher risk of developing breast cancer. Our failure to find associations in postmenopausal women is in agreement with Bertone-Johnson et al [7] and Vachon et al [8] for vitamin D intake and with several authors [8-11,13,33] for circulating levels of vitamin D. The outcomes of our study should be considered preliminary because of the following limitations: the sample size, cross-sectional nature of the study, and limited generalizability due to recruitment from a single mammography center. In addition, a single serum measure of vitamin D might not accurately classify subjects with respect to their usual vitamin D status.

The association of vitamin D and breast density remains unclear perhaps due to misclassification of dietary intake and the varying ability of some persons to absorb vitamin D through sunlight [4]. Confirmation of our findings in larger longitudinal studies may assist in clarifying the role of vitamin D in breast density over a longer duration of time.

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